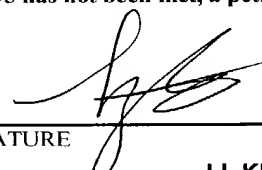


FORM PTO-1390 (Modified) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV. 10-95)		ATTORNEY'S DOCKET NUMBER BB-1262
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR) 09/857612
INTERNATIONAL APPLICATION NO. PCT/US99/28586	INTERNATIONAL FILING DATE 2 DECEMBER 1999 (02.12.999)	PRIORITY DATE CLAIMED 3 DECEMBER 1998 (03.12.98)
TITLE OF INVENTION PLAN LECITHIN:CHOLESTEROL ACYLTRANSFERASES		
APPLICANT(S) FOR DO/EO/US CAHOON, Rebecca E., et al.		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information		
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This is an express request to being national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b)) and PCT Articles 22 and 39(1). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 5. <input checked="" type="checkbox"/> A copy of the International Application was filed (35 U.S.C. 371 (c) (2)) <ol style="list-style-type: none"> a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau. b. <input type="checkbox"/> has been transmitted by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US) 6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371 (c) (2)). 7. <input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210). 8. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c) (3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 9. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)). 10. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). 11. <input checked="" type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409) 12. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)). 		
Items 13 to 18 below concern document(s) or information included :		
<ol style="list-style-type: none"> 13. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 14. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 15. <input checked="" type="checkbox"/> A FIRST preliminary amendment. A SECOND or SUBSEQUENT preliminary amendment. 16. <input type="checkbox"/> A substitute specification. 17. <input checked="" type="checkbox"/> A change of power of attorney and/or address letter. 18. <input checked="" type="checkbox"/> Certificate of Mailing by Express Mail. 19. <input type="checkbox"/> Other items or information: 		
<div style="border: 1px solid black; padding: 5px;"> <p>17. General Power of Attorney</p> <p>18. Express Mailing Label No.: EL031052612US</p> </div>		

531 Rec'd PCT 04 JUN 2001

APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.53) INTERNATIONAL APPLICATION NO. PCT/US99/28586				ATTORNEY'S DOCKET NUMBER BB-1262	
20. The following fees are submitted				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :					
<input checked="" type="checkbox"/> Search Report has been prepared by the EPO or JPO \$860.00					
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) \$690.00					
<input type="checkbox"/> No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$760.00					
<input type="checkbox"/> Neither international preliminary examination fee paid to USPTO (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$1000.00					
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) And all claims satisfied provisions of PCT Article 33(2)-(4) \$ 100.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$860.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)). <input type="checkbox"/> 20 <input type="checkbox"/> 30				\$0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	17 - 20 =	0 x	\$18.00	\$0.00	
Independent Claims	2 - 3 =	0 x	\$80.00	\$ 0	
Multiple Dependent Claims (check if applicable)			<input type="checkbox"/>	\$ 0	
TOTAL OF ABOVE CALCULATIONS =				\$ 0	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable). <input type="checkbox"/>				\$0.00	
SUBTOTAL =				\$ 0	
Processing Fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)). <input type="checkbox"/> 20 <input type="checkbox"/> 30				\$0.00	
TOTAL NATIONAL FEE =				\$ 860	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). <input type="checkbox"/>				\$0.00	
TOTAL FEES ENCLOSED =				\$ 860	
				Amount to be : refunded	\$
				Charged	\$
<input type="checkbox"/> A check in the amount of _____ to cover the above fees enclosed.					
<input checked="" type="checkbox"/> Please charge my Deposit Account No. 04-1928 in the amount of \$ 860.00 to cover the above fees.					
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 04-1928 a duplicate copy of this sheet is enclosed.					
NOTE : Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (CFR 1.37(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO:					
LI, Kening E. I. DU PONT DE NEMOURS AND COMPANY Legal Patent Records Center 1007 Market Street Wilmington, Delaware 19898 United States of America			SIGNATURE  NAME LI, KENING REGISTRATION NUMBER 44,872 DATE 06/04/2001		

13 Rec'd PCT/PTO 18 OCT 2001

09/857612

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:

E. I. du Pont de Nemours and Company

CASE NO.: BB1262

APPLICATION NO.: 09/857612

GROUP ART UNIT: UNKNOWN

FILED: JUNE 4, 2001

EXAMINER: UNKNOWN

FOR: PLANT LECITHIN: CHOLESTEROL
ACYLTRANSFERASES

STATEMENT UNDER 37 CFR 1.821(g) and 1.825(b)

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

The submission of the substitute Sequence Listing filed concurrently herewith does not include new matter.

The copy of the substitute Sequence Listing in computer readable form filed concurrently herewith is the same as the paper copy of the substitute Sequence Listing filed concurrently herewith.

Respectfully submitted,



Paul D. Golian
Attorney For Applicants
Registration No. 42,591
Telephone: 302-992-3749
Facsimile: 302-892-1026

Dated: October 15, 2001

098376109/857612

531 Rec'd PCT

04 JUN 2001
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
K. BUTLER ET AL.

CASE NO.: BB1262

APPLICATION NO.: UNKNOWN

GROUP ART UNIT: UNKNOWN

FILED: CONCURRENTLY HEREWITH

EXAMINER: UNKNOWN

FOR: UDP-GLUCOSE MODIFIERS

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Before examination of the above-referenced application, please amend the application as follows:

In the Claims:

Please cancel claims 1-18.

Please add the following new claims:

- 19. An isolated polynucleotide that encodes a plant lecithin:cholesterol acyltransferases polypeptide having a sequence identity of at least 80%, based on the Clustal method of alignment, when compared to a polypeptide selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12 and 14.
- 20. The polynucleotide of Claim 19 wherein the sequence identity is at least 85%.
- 21. The polynucleotide of Claim 19 wherein the sequence identity is at least 90%.
- 22. The polynucleotide of Claim 19 wherein the sequence identity is at least 95%.
- 23. The polynucleotide of Claim 19 wherein the polypeptide is selected from the group consisting of SEQ ID Nos: 2, 4, 6, 8, 10, 12, and 14.
- 24. The polynucleotide of Claim 19, wherein the polynucleotide is selected from SEQ ID Nos: 1, 3, 5, 7, 9, 11, and 13.
- 25. An isolated complement of the polynucleotide of Claim 19, wherein (a) the complement and the polynucleotide consist of the same number of nucleotides, and (b) the nucleotide sequences of the complement and the polynucleotide have 100% complementarity.

Application No.: Unknown

Docket No.: BB1262

Page 2

26. An isolated nucleic acid molecule that encodes a UDP glucose pyrophosphorylase polypeptide and remains hybridized with the isolated polynucleotide of Claim 19 under a wash condition of 0.1X SSC, 0.1% SDS, and 65°C.
27. A cell or a virus comprising the polynucleotide of Claim 19.
28. The cell of Claim 27 wherein the cell is selected from the group consisting of a yeast cell, a bacterial cell, an insect cell, and a plant cell.
29. A transgenic plant comprising the polynucleotide of Claim 19.
30. A method for transforming a cell comprising introducing into a cell the polynucleotide of Claim 19.
31. A method for producing a transgenic plant comprising (a) transforming a plant cell with the polynucleotide of Claim 19, and (b) regenerating a plant from the transformed plant cell.
32. An isolated a plant lecithin:cholesterol acyltransferases polypeptide having a sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, 10, 12 and 14.
33. A chimeric gene comprising the polynucleotide of Claim 19 operably linked to at least one suitable regulatory sequence.
34. The chimeric gene of Claim 33, wherein the chimeric gene is an expression vector.
35. A method for altering the level of plant lecithin:cholesterol acyltransferases polypeptide expression in a host cell, the method comprising:
 - (a) Transforming a host cell with the chimeric gene of claim 33; and
 - (b) Growing the transformed cell in step (a) under conditions suitable for the expression of the chimeric gene. --

Application No.: Unknown

Docket No.: BB1262

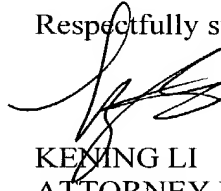
Page 3

Remarks

Applicants respectfully submit that newly added claims more clearly and distinct recite that which applicants consider to be their invention, and are adequately supported by the original disclosure.

No new matter is believed to be at issue. Entry of the amendments and early favorable consideration of the claims on the merits are hereby respectfully requested.

Respectfully submitted,



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Dated: 06/04/2001

TITLEPLANT LECITHIN:CHOLESTEROL ACYLTRANSFERASES

This application claims the benefit of U.S. Provisional Application No. 60/110,782, filed December 3, 1998.

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding plant lecithin:cholesterol acyltransferases in plants and seeds.

BACKGROUND OF THE INVENTION

Phosphatidylcholine-sterol O-acyltransferase (EC 2.3.1.43) transfers acyl groups from phosphatidylcholine to sterols. This enzyme is also known as lecithin:cholesterol acyltransferase (LCAT) and belongs to the class of CoA-independent acyltransferases. This enzyme is found associated with high-density lipoproteins (HDL) and forming a complex with its activators Apolipoprotein (apo)-AI and apo-D. HDLs are believed to promote the process of reverse cholesterol transport. This process involves efflux of cellular cholesterol, cholesterol esterification and lipid transport and exchange. Apo A-I and lecithin-cholesterol acyltransferase play a crucial role in reverse cholesterol transport.

The role of LCAT in plants will presumably be different from mammalian systems given the negligible levels of cholesterol found in plant oils. However, plants have a complex combination of membrane sterols that can change with environmental conditions as well as developmental determination. LCAT may function as the phosphatidylcholine acyl-exchange enzyme which moves unsaturated acyl groups into phosphatidylcholine for desaturation and out of it for incorporation into triacylglycerols. Overexpression of LCAT may lead to increased lipid metabolism and fluidity of membranes increasing resistance to heat and/or cold shock. Overexpression or cosuppression of LCAT may also be useful to genetically alter the content of phytosterol or lecithin in grains.

SUMMARY OF THE INVENTION

The present invention relates to isolated polynucleotides comprising a nucleotide sequence encoding a first polypeptide of at least 417 amino acids that has at least 60% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a corn plant lecithin:cholesterol acyltransferases polypeptide of SEQ ID NOs:2, 4, 8, 10, or 12, and a soybean plant lecithin:cholesterol acyltransferases polypeptide of SEQ ID NOs:6, or 14. The present invention also relates to an isolated polynucleotide comprising the complement of the nucleotide sequences described above.

It is preferred that the isolated polynucleotides of the claimed invention consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, and 14. The present invention also relates to an isolated polynucleotide

comprising a nucleotide sequences of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13 and the complement of such nucleotide sequences.

5 The present invention relates to an isolated polynucleotide comprising at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13 and the complement of such sequences.

 The present invention relates to a chimeric gene comprising an isolated polynucleotide of the present invention operably linked to suitable regulatory sequences.

10 The present invention relates to an isolated host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention. The host cell may be eukaryotic, such as a yeast or a plant cell, or prokaryotic, such as a bacterial cell. The present invention also relates to a virus, preferably a baculovirus, comprising an isolated polynucleotide of the present invention or a chimeric gene of the present invention.

15 The present invention relates to a process for producing an isolated host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention, the process comprising either transforming or transfecting an isolated compatible host cell with a chimeric gene or isolated polynucleotide of the present invention.

 The present invention relates to a plant lecithin:cholesterol acyltransferases polypeptide of at least 417 amino acids comprising at least 60% homology based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, and 14.

20 The present invention relates to a method of selecting an isolated polynucleotide that affects the level of expression of a plant lecithin:cholesterol acyltransferases polypeptide in a host cell, preferably a plant cell, the method comprising the steps of:

 constructing an isolated polynucleotide of the present invention or an isolated chimeric gene of the present invention;

 introducing the isolated polynucleotide or the isolated chimeric gene into a host cell;

30 measuring the level a plant lecithin:cholesterol acyltransferases polypeptide in the host cell containing the isolated polynucleotide; and

 comparing the level of a plant lecithin:cholesterol acyltransferases polypeptide in the host cell containing the isolated polynucleotide with the level of a plant lecithin:cholesterol acyltransferases polypeptide in a host cell that does not contain the isolated polynucleotide.

35 The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a lecithin:cholesterol acyltransferases polypeptide gene, preferably a plant plant lecithin:cholesterol acyltransferases polypeptide gene, comprising

the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13 and the complement of such nucleotide sequences; and
5 amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a plant lecithin:cholesterol acyltransferases amino acid sequence.

The present invention also relates to a method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a plant
10 lecithin:cholesterol acyltransferases polypeptide comprising the steps of: probing a cDNA or genomic library with an isolated polynucleotide of the present invention; identifying a DNA clone that hybridizes with an isolated polynucleotide of the present invention; isolating the identified DNA clone; and sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of a plant lecithin:cholesterol acyltransferases, the method comprising the steps of: (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a plant lecithin:cholesterol acyltransferases, operably linked to suitable regulatory sequences; (b) growing the transformed host cell under
20 conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of plant lecithin:cholesterol acyltransferases in the transformed host cell; (c) optionally purifying the plant lecithin:cholesterol acyltransferases expressed by the transformed host cell; (d) treating the plant lecithin:cholesterol acyltransferases with a compound to be tested; and (e) comparing the activity of the plant
25 lecithin:cholesterol acyltransferases that has been treated with a test compound to the activity of an untreated plant lecithin:cholesterol acyltransferases, thereby selecting compounds with potential for inhibitory activity.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE DESCRIPTIONS

30 The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application.

Figure 1 depicts the amino acid sequence alignment between the lecithin:cholesterol acyltransferase corn clone cen3n.pk0141.f2:fis (SEQ ID NO:8), corn clone cep1c.pk001.f7:fis (SEQ ID NO:10), corn clone chpc24.pk0001.c1 (SEQ ID NO:12), and
35 soybean clone sl2.pk0015.e8:fis (SEQ ID NO:14), with the *Arabidopsis thaliana* lecithin:cholesterol acyltransferase protein (NCBI General Identifier No. 3935185; SEQ ID NO:15). Conserved sequence elements are shown boxed in black with the amino acid sequence in white. The second boxed sequence (HS*G) contains a conserved serine that is

believed to be the active site residue found in all serine lipases. This sequence motif is also conserved in mammalian lecithin:cholesterol acyltransferases (Rogne et al. (1987) *Biochem Biophys Res Commun* 148:161-169). All of the boxed sequences are largely conserved in the mammalian lecithin:cholesterol acyltransferase sequences.

Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1
Lecithin:Cholesterol Acyltransferases

Lecithin:Cholesterol Acyltransferase (LCAT)	Clone Designation	SEQ ID NO:	
		(Nucleotide)	(Amino Acid)
maize [Zea mays]	cep1c.pk001.f7	1	2
maize [Zea mays]	Contig of: chpc24.pk0001.c1 cbn2.pk0017.h4 cbn10.pk0052.g10 p0016.ctsbo30r p0018.chstj36r	3	4
soybean [Glycine max]	sl2.pk0015.e8	5	6
maize [Zea mays]	cen3n.pk0141.f2: fis	7	8
maize [Zea mays]	cep1c.pk001.f7: fis	9	10
maize [Zea mays]	chpc24.pk0001.c1	11	12
soybean [Glycine max]	sl2.pk0015.e8: fis	13	14

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res.* 13:3021-3030 (1985) and in the *Biochemical J.* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. As used herein, a "polynucleotide" is a nucleotide sequence such as a nucleic acid fragment. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA,

synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least one of 60 contiguous nucleotides, preferably at least one of 40 contiguous nucleotides, most preferably one of at least 30 contiguous nucleotides, of the nucleic acid sequence of the SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, or the complement of such sequences.

As used herein, "contig" refers to a nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share common or overlapping regions of sequence homology. For example, the nucleotide sequences of two or more nucleic acid fragments can be compared and aligned in order to identify common or overlapping sequences. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be assembled into a single contiguous nucleotide sequence.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof.

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell. For example, a substantially similar nucleic acid fragment representing at least one of 30 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than

the entire coding region of a gene, and by nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13 and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of a polypeptide (such as lecithin:cholesterol acyltransferases) in a host cell. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a host cell (eukaryotic, such as plant or yeast, prokaryotic such as bacterial, or viral) may comprise the steps of: constructing an isolated polynucleotide of the present invention or an isolated chimeric gene of the present invention; introducing the isolated polynucleotide or the isolated chimeric gene into a host cell; measuring the level a polypeptide in the host cell containing the isolated polynucleotide; and comparing the level of a polypeptide in the host cell containing the isolated polynucleotide with the level of a polypeptide in a host cell that does not contain the isolated polynucleotide.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) Nucleic Acid Hybridisation, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with

of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. "Chemically synthesized", as related to nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature.

Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature.

“Endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign” gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A “transgene” is a gene that has been introduced into the genome by a transformation procedure.

“Coding sequence” refers to a nucleotide sequence that codes for a specific amino acid sequence. “Regulatory sequences” refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

“Promoter” refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an “enhancer” is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”. New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

The “translation leader sequence” refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol.* 3:225-236).

The "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell* 1:671-680.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into polypeptide by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide by the cell. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term "operably linked" refers to the association of two or more nucleic acid fragments on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

"Altered levels" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

“Mature” protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. “Precursor” protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A “chloroplast transit peptide” is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. “Chloroplast transit sequence” refers to a nucleotide sequence that encodes a chloroplast transit peptide. A “signal peptide” is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

“Transformation” refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as “transgenic” organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or “gene gun” transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference).

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter “Maniatis”).

Nucleic acid fragments encoding at least a portion of several plant lecithin:cholesterol acyltransferases have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other plant lecithin:cholesterol acyltransferases, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673-5677; Loh et al. (1989) *Science* 243:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably one of at least 40, most preferably one of at least 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide. The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a polypeptide of a gene (such as plant lecithin:cholesterol acyltransferases) preferably a substantial portion of a plant polypeptide of a gene, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of

60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the
5 oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a polypeptide.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be
10 used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol.* 36:1-34; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic
15 plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of, and composition of, plant sterols in those cells. Since plant sterols help regulate membrane fluidity, this may be beneficial in regulating cold or heat tolerance in plants. Also, it is believed that lecithin is the acyl donor for this reaction,
20 therefore the levels of lecithin found in the plants could be altered by varying the activity of LCAT.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of
25 development. For reasons of convenience, the chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant chimeric gene can then be constructed. The
30 choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al.
35 (1985) *EMBO J.* 4:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of

DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant polypeptide to different cellular compartments, or to facilitate its secretion from the cell. It is thus envisioned that the chimeric gene described above may be further supplemented by altering the coding sequence to encode the instant polypeptide with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell* 56:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.* 100:1627-1632) added and/or with targeting sequences that are already present removed. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of utility may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants for some applications. In order to accomplish this, a chimeric gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Alternatively, a chimeric gene designed to express antisense RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U.S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In addition, the ability to restrict the expression of specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above,

it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on practical grounds, and is not an inherent part of the invention. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one which allows large numbers of samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired phenotype.

The instant polypeptide (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptide of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptide are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptide. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded plant lecithin:cholesterol acyltransferases. An example of a vector for high level expression of the instant polypeptide in a bacterial host is provided (Example 6).

Additionally, the instant polypeptide can be used as a targets to facilitate design and/or identification of inhibitors of those enzymes that may be useful as herbicides. This is desirable because the polypeptide described herein catalyze steps in sterol modification in plants. The composition of plant sterols are important factors in a plants ability to adapt to temperature changes in the environment, shifts in sunlight, and drought stress. In addition, the sterol derivative hormones, such as brassinosteroids, can affect overall growth and development of plants (Szekeres et al. (1996) *Cell* 85:171-182; Clouse and Sasse (1998) *Ann Rev Plant Physiol Plant Mol Biol* 49:427-451). Brassinosteroid production may be affected by changes in composition of membrane sterols. Accordingly, inhibition of the activity of one or more of the enzymes described herein could lead to inhibition of plant growth. Thus, the instant polypeptide could be appropriate for new herbicide discovery and design.

All or a substantial portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with

the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics* 1:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet.* 32:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter* 4:37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan et al. (1995) *Genome Res.* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 11:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet.* 7:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA* 86:9402-9406; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* 92:8149-8153; Bensen et al. (1995) *Plant Cell* 7:75-84). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptide. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptide can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptide disclosed herein.

EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various corn and soybean tissues were prepared. The characteristics of the libraries are described below.

TABLE 2
cDNA Libraries from Corn and Soybean

Library	Tissue	Clone
cbn10	Corn Developing Kernel 10 Days After Pollination	cbn10.pk0052.g10
cbn2	Corn Developing Kernel Two Days After Pollination	cbn2.pk0017.h4
p0016	Corn Embryo 13 Days After Pollination	p0016.ctsbo30r
p0018	Corn Ear Shoot	p0018.chstj36r
cen3n	Corn Endosperm 20 Days After Pollination*	cen3n.pk0141.f2:fis
ceplc	Corn (<i>Zea mays</i> L.) pollinated (25 hrs after pollination, 48-72 after emergence) ears	ceplc.pk001.f7:fis
chpc24	Corn (MBS847) 8 Day Old Shoot Treated 24 Hours With PDO Herbicide MK593**	chpc24.pk0001.c1
sl2	Soybean Two-Week-Old Developing Seedlings Treated With 2.5 ppm chlorimuron	sl2.pk0015.e8:fis

*This library was normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

5 **Application of 2-[(2,4-dihydro-2,6,9-trimethyl[1]benzothiopyrano[4,3-c]pyrazol-8-yl)carbonyl]-1,3-cyclohexanedione *S,S*-dioxide; synthesis and methods of using this compound are described in WO 97/19087, incorporated herein by reference.

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into pre-cut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science* 252:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

EXAMPLE 2

Identification of cDNA Clones

cDNA clones encoding plant lecithin:cholesterol acyltransferases were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.*

215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet.* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

EXAMPLE 3

Characterization of cDNA Clones Encoding Lecithin:Cholesterol Acyltransferases

The BLASTX search using the nucleotide sequences from clones cep1c.pk001.f7 and sl2.pk0015.e8 and the nucleotide sequences from the contig assembled of clones chpc24.pk0001.c1, cbn2.pk0017.h4, cbn10.pk0052.g10, p0016.ctsbo30r and p0018.chstj36r revealed similarity of the proteins encoded by the cDNAs to lecithin:cholesterol acyltransferase from *Homo sapiens* (NCBI gi Accession No. 998999) and from *rattus norvegicus* (NCBI gi Accession No. 418623). The BLAST results for each of these sequences are shown in Table 3:

TABLE 3

BLAST Results for Clones Encoding Polypeptides Homologous
to Lecithin:Cholesterol Acyltransferases

Clone	Status	Blast pLog Score	
		98999	418623
cep1c.pk001.f7	EST	16.40	13.40
Contig of:	Contig	12.10	14.00
chpc24.pk0001.c1			
cbn2.pk0017.h4			
cbn10.pk0052.g10			
p0016.ctsbo30r			
p0018.chstj36r			
sl2.pk0015.e8	EST	44.00	42.05

The sequence of a portion of the cDNA insert from clone cep1c.pk001.f7 is shown in SEQ ID NO:1; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:2.

The sequence of a contig assembled of the cDNA inserts from clones chpc24.pk0001.c1, cbn2.pk0017.h4, cbn10.pk0052.g10, p0016.ctsbo30r and p0018.chstj36r is shown in SEQ ID NO:3; the deduced amino acid sequence of this contig is shown in SEQ ID NO:4. The sequence of the entire cDNA insert in clone sl2.pk0015.e8 was determined and is shown in SEQ ID NO:5; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:6. The amino acid sequence set forth in SEQ ID NO:6 was evaluated by BLASTP, yielding a pLog value of 47.30 versus the *Homo sapiens* sequence. The soybean lecithin:cholesterol acyltransferase is 32.2% identical to the *Homo sapiens* sequence. Sequence percent identity calculations were performed by the Jotun Hein method (Hein, J. J. (1990) *Meth. Enz.* 183:626-645) using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI) with the default parameters for pairwise alignment (ktuple=2). BLAST scores and probabilities indicate that the instant nucleic acid fragments encode portions of corn lecithin:cholesterol acyltransferase and an almost entire soybean lecithin:cholesterol acyltransferases. These sequences represent the first plant sequences encoding lecithin:cholesterol acyltransferases.

After the filing of the provisional application (U.S. Provisional Application No. 60/110782, filed December 3, 1998) an *Arabidopsis thaliana* gene was discovered in the NCBI database (with a deposit date of December 1, 1998). The BLASTX search using the EST sequences from clones listed in Table 4 revealed similarity of the polypeptides encoded by the cDNAs to lecithin:cholesterol acyltransferases from *Arabidopsis thaliana* (NCBI Accession No. gi 3935185). Shown in Table 4 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), contigs assembled from two or more ESTs ("Contig"), contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 4
BLAST Results for Sequences Encoding Polypeptides Homologous
to Lecithin:Cholesterol Acyltransferases

Clone	Status	BLAST pLog Score 3935185
cen3n.pk0141.f2:fis	CGS	63.70
cep1c.pk001.f7:fis	CGS	84.00
chpc24.pk0001.c1	FIS	64.52
sl2.pk0015.e8:fis	CGS	152.00

Figure 1 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:8, 10, 12, and 14, and the *Arabidopsis thaliana* sequence (SEQ ID NO:15). The data in

Table 4 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:8, 10, 12, and 14, and the *Arabidopsis thaliana* sequence (SEQ ID NO:15).

TABLE 4

5 Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Lecithin:Cholesterol Acyltransferases

SEQ ID NO.	Percent Identity to 3935185
8	29.4%
10	37.2%
12	28.5%
14	57.6%

Sequence alignments and percent identity calculations were performed using the
10 Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3,
15 WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a lecithin:cholesterol acyltransferases. These sequences represent the first plant sequences encoding lecithin:cholesterol acyltransferases.

EXAMPLE 4

20 Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptide in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain
25 reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate
30 band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from

pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptide, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 µm in diameter) are coated with DNA using the following technique. Ten µg of plasmid DNAs are added to 50 µL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 µL of a 2.5 M solution) and spermidine free base (20 µL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 µL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 µL of ethanol. An aliquot (5 µL) of the DNA-coated

gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

5 For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a
10 helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains gluphosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to
15 fresh N6 medium containing gluphosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of
20 tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* 8:833-839).

EXAMPLE 5

Expression of Chimeric Genes in Dicot Cells

25 A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant polypeptide in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about
30 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction
35 (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described

retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 6

Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptide can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 µg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 µL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the instant polypeptide

the use of an affinity resin which is specific for the fusion protein tag attached to the expressed enzyme or an affinity resin containing ligands which are specific for the enzyme. For example, the instant polypeptide may be expressed as a fusion protein coupled to the C-terminus of thioredoxin. In addition, a (His)₆ peptide may be engineered into the N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be accomplished using other reagents which interact to displace the thioredoxin from the resin. These reagents include β -mercaptoethanol or other reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the enzyme may be accomplished after the fusion protein is purified or while the protein is still bound to the ThioBond™ affinity resin or other resin.

Crude, partially purified or purified enzyme, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit enzymatic activation of the instant polypeptide disclosed herein. Assays may be conducted under well known experimental conditions which permit optimal enzymatic activity. For example, assays for lecithin:cholesterol acyltransferases are presented by Manabe, M. et al. (1987) *J. Lipid Res.* 28:1206-1215.

Various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth above is incorporated herein by reference in its entirety.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/54, 15/82, 9/10, C12Q 1/02, 1/48, 1/68	A3	(11) International Publication Number: WO 00/32791	(43) International Publication Date: 8 June 2000 (08.06.00)
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(21) International Application Number: PCT/US99/28586
(22) International Filing Date: 2 December 1999 (02.12.99)
(30) Priority Data:
60/110,782 3 December 1998 (03.12.98) US

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(81) Designated States: AE, AL, AU, BA, BB, BG, BR, CA, CN,
CR, CU, CZ, DM, EE, GD, GE, HR, HU, ID, IL, IN, IS,
JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX,
NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ,
VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD,
SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG,
KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY,
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT,
SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW,
ML, MR, NE, SN, TD, TG).

Published

With international search report.

(88) Date of publication of the international search report:
14 September 2000 (14.09.00)

(54) Title: PLAN LECITHIN:CHOLESTEROL ACYLTRANSFERASES

(57) Abstract

This invention relates to an isolated nucleic acid fragment encoding a plant lecithin:cholesterol acyltransferases. The invention also relates to the construction of a chimeric gene encoding all or a portion of the plant lecithin:cholesterol acyltransferases, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the plant lecithin:cholesterol acyltransferases in a transformed host cell.

Arabidopsis (gi 3935185) MKK-----1-SSHSVVIALLVVVTMTSMCAVGSN-----VYPLILVPGNGGQLEVR
corn_ (SEQ ID NO:2) HAR-IPVLAPELLLLPAGLR--ELMIDRRPLPKRCRREVLHPLVLVPLGTCSELDAR
corn_ (SEQ ID NO:4) MVNDHAS-----CSRGGTIVLSKFASTTRAPKO-----LPPVVVVPGVATNELDAR
corn_ (SEQ ID NO:6) NASSLLQOLLSSLLLLPSPLRLREHLSGNHAYSAN-----NFHPIFLVAGVSCSDEAR
soybean_ (SEQ ID NO:8) HMKLEELGL-KIEVATLTVTVVVVHLSLCTCGASN-----LDPLILPGNGGQLEVR
Arabidopsis (gi 3935185) LDREYKPSVSMCSWLYPIHKKSGGMFLWFDAAVLLSPFT--RCFSDRMHLYDPDLDDY
corn_ (SEQ ID NO:2) L7DAYAFFRAACDE-----GGLVRLMTNCSDLPAHNYVRCFMEQMALYDVPVANDY
corn_ (SEQ ID NO:4) LTELHPSSPRCA-----HKGKGMFLRYLNTYALEDAADVRCFAEQMATAYDAASDDY
corn_ (SEQ ID NO:6) LTELHPSSPRCA-----HKGKGMFLRYLNTYALEDAADVRCFAEQMATAYDAASDDY
soybean_ (SEQ ID NO:8) L7NOYKPSFTICESW-YPLIKKNGMFLWFDSSVILAPFT--OCTAERMTLHYHQELDDY
Arabidopsis (gi 3935185) QNAPGVOTRVPVHFGSTKSLYLDPRLRDTATSYHEHLVKALEKCCGVYNDOTIL
corn_ (SEQ ID NO:2) RNLPGVETVPVHFGSRRGF-DKNPEHTTSMCEFLVRLNELARA-GYRGGDTLFL
corn_ (SEQ ID NO:4) RNAQGVETVPVHFGSTRAPYDPPDRHNF--SYMDFVSRLERL--AYRDGENLFL
corn_ (SEQ ID NO:6) RNLAGVETVPVHFGSTRAPYDPPDRHNF--SYMDFVSRLERL--AYRDGENLFL
soybean_ (SEQ ID NO:8) FNTFGVETVPVHFGSTNSLLYLNLPRKHTIGYMAPLVDSLQKL--GYADGETLFL
Arabidopsis (gi 3935185) GLAASGHPSRVASQFLQDLKQVLEKTSSENEGKPVILL
corn_ (SEQ ID NO:2) APPVPGQPSRSPATSVGMPSLVEDASRKNRGRKVLFLG
corn_ (SEQ ID NO:4) AVAPPGHPSRVADAFGLRLRLVERASRANGGGPVTVIA
corn_ (SEQ ID NO:6) APPSPGQTSSEVYSRYFKELMELVEAASERTR--KKAVALIG
soybean_ (SEQ ID NO:8) GLAEGHPSOVGSKFLKDLKNTLEASNSNNGKPVILL
Arabidopsis (gi 3935185) RYIKHFLVALAAPH--GCTISQMTFASGNTLG-VPLVNL--LVRRHRTSSEHOMLLPS
corn_ (SEQ ID NO:2) DRYIKHFLVALAAPH--GCTISQMTFASGNTLG-VPLVNL--LVRRHRTSSEHOMLLPS
corn_ (SEQ ID NO:4) RYIKHFLVALAAPH--GCTISQMTFASGNTLG-VPLVNL--LVRRHRTSSEHOMLLPS
corn_ (SEQ ID NO:6) RYIKHFLVALAAPH--GCTISQMTFASGNTLG-VPLVNL--LVRRHRTSSEHOMLLPS
soybean_ (SEQ ID NO:8) RYIKHFLVALAAPH--GCTISQMTFASGNTLG-VPLVNL--LVRRHRTSSEHOMLLPS
Arabidopsis (gi 3935185) TKVHHDRTKPLVVTPOVNYTA--YENDRFADIGTSQGVYFVYKTRVLPLTEELMTPOVNV
corn_ (SEQ ID NO:2) PAVFGH--RPLVVTARRNYSA--YDLEBLVAVGVGACVPEFRRARVPHNSYFGAPVVPVT
corn_ (SEQ ID NO:4) PAVFGH--RPLVVTARRNYSA--YDLEBLVAVGVGACVPEFRRARVPHNSYFGAPVVPVT
corn_ (SEQ ID NO:6) PAVFGH--RPLVVTARRNYSA--YDLEBLVAVGVGACVPEFRRARVPHNSYFGAPVVPVT
soybean_ (SEQ ID NO:8) PAVFGH--RPLVVTARRNYSA--YDLEBLVAVGVGACVPEFRRARVPHNSYFGAPVVPVT
Arabidopsis (gi 3935185) TCIIY KOPEIK- ASLAAL-----KVDS-LN
corn_ (SEQ ID NO:2) TCMN ATPEIV- VSMIAFDEKMRROPEQNKVYK
corn_ (SEQ ID NO:4) ACVV VTPMNV- VSLLAQDP-AMRLPTA-Y-FR
corn_ (SEQ ID NO:6) TYIS AAPEVAA- ISVLAFAKEHMRROPEQNKVYK
soybean_ (SEQ ID NO:8) TCIM ERPEIS- VSLLAQDP-AMRLPTA-Y-FR
Arabidopsis (gi 3935185) TVEIDGVSHSTILKDEALKEIMKQISIIY--ELANVNAVNE
corn_ (SEQ ID NO:2) SIKIRGAQHGTVTDUTLAKRVHHEILEANR-----S
corn_ (SEQ ID NO:4) HLKVRNVSHSTILKDEALKEIMKQISIIY--ELANVNAVNE-----LRPN
corn_ (SEQ ID NO:6) SIKIRGAQHGTVTDUTLAKRVHHEILEANR-----S
soybean_ (SEQ ID NO:8) VVKIDGVSHSTILKDEALKEIMKQISIIY--ELANVNAVNE-----LRPN

Docket Number
BB1262PCT

DECLARATION and POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PLANT LECITHIN: CHOLESTEROL ACYLTRANSFERASES

the specification of which is attached hereto unless the following box is checked:

☒ was filed on **02 DECEMBER 1999** as U.S. Application No. _____ or PCT International Application No. **PCT/US99/28586** and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Application No.	Country	Filing Date	Priority Claimed (Yes/No)

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States Provisional Application(s) listed below.

U.S. Provisional Application No.

60/110,782

U.S. Filing Date

03 DECEMBER 1998

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International Application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application or PCT International Application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application No.	Filing Date	Status (patented, pending or abandoned)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Frank, George A.	<u>27,636</u>	Shay, Lucas K.	<u>34,724</u>
Golian, Andrew G.	<u>25,293</u>	Shipley, James E.	<u>32,003</u>
Gorman, Thomas W.	<u>31,959</u>	Siegeil, Barbara C.	<u>30,684</u>
Gould, David J.	<u>25,338</u>	Sinnott, Jessica M.	<u>34,015</u>
Griffiths, John E.	<u>32,647</u>	Steinberg, Thomas W.	<u>37,013</u>
Hamby, Jane O.	<u>32,872</u>	Stevenson, Robert B.	<u>26,039</u>
Hamby, William H.	<u>31,521</u>	Strickland, Frederick D.	<u>39,041</u>
Heiser, David E.	<u>31,366</u>	Tessari, Joseph A.	<u>32,177</u>
Hendrickson, John S.	<u>30,847</u>	Tulloch, Rebecca W.	<u>36,297</u>
Jones, Brian C.	<u>37,857</u>	Walker, P. Michael	<u>32,602</u>
Joung, J. Kenneth	<u>41,881</u>	Wang, Chen	<u>38,650</u>

The undersigned ratifies fully all actions already taken by the above-named individuals in accordance with the authority granted hereby.

E. I. DU PONT DE NEMOURS AND COMPANY

By: Vernon R. Rice

Vernon R. Rice

Vice President and Assistant General Counsel

Date: 5/11/2000

SEQUENCE LISTING

<110> E. I. du Pont de Nemours and Company
<120> Plant Lecithin:Cholesterol Acyltransferases
<130> BB1262
<140> US/09/857,612
<141> 2001-07-19
<150> 60/110,782
<151> 1998-12-03
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gggttgggct ggncacgccg ggaanatgct ggcctaaccg gggaagacga anttcgacgt 480
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ct 542

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<210> 2

<211> 143

<212> PRT

<213> Zea mays

<400> 2

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          20           25           30
Ala Ala Pro Trp Gly Gly Val Val Leu Gly Met Leu Thr Ile Val Ala
          35           40           45
Gly Asn Asn Leu Gly Leu Pro Phe Val Asp Pro Leu Ala Leu Lys Gly
          50           55           60
Glu Tyr Arg Ser Leu Gln Ser Ser Leu Trp Pro Leu Pro Asn Pro Asn
          65           70           75           80
Ala Phe Arg Ala Gly Gln Pro Leu Val Thr Thr Arg Ser Arg Thr Tyr
          85           90           95
Thr Ala His Asp Met Ala Asp Phe Leu Asp Ala Ile Gly Leu Gly Ala
          100          105          110
Ala Ile Val Pro Tyr Gln Ser Arg Val Leu Pro Leu Phe Arg Glu Leu
          115          120          125
Pro Ser Pro Arg Val Pro Val Ala Cys Val Arg Pro Gly Leu Gly
          130          135          140

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<211> 921

<212> DNA

<213> Zea mays

<220>

<221> unsure

<222> (884)

<223> n=A, C, G, or T

<400> 3

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ttccacccca tctttctggt agctggggtg agctgcagcg acctggaggc acgcctcacc 240
gaggagtacc ggccgtcggg gccgcactgc ggcgccatga aggggaaggg gtgggttcggt 300
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cgagtgcaca acttcggctc cacaagagcc ttcagccaca agaaccacct caagtcagac 480
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atgttcggag cccctacga ctccgctac gcgcgcgct ccccgccga gacgtccgag 600
gtgtactccc gctacttcaa ggagctgatg gagctggtcg aggcgcgag cgagaggacc 660
cggaagaagg ccgtcatcct cggccacagc ttccggcgga tggtcgcgct cgagttcgtc 720
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<213> Zea mays

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<400> 4

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  1              5              10              15

```

```

Leu Pro Ser Pro Leu Arg Leu Arg Glu His Leu Ser Gly Asn His Ala
          20              25              30

```

```

Val Ser Ala Asn Asn Phe His Pro Ile Phe Leu Val Ala Gly Val Ser
      35              40              45

```

```

Cys Ser Asp Leu Glu Ala Arg Leu Thr Glu Glu Tyr Arg Pro Ser Val
      50              55              60

```

```

Pro His Cys Gly Ala Met Lys Gly Lys Gly Trp Phe Gly Leu Trp Lys
      65              70              75              80

```

```

Asn Ser Ser Glu Leu Leu Ser Arg Asp Tyr Val Gln Cys Phe Glu Glu
          85              90              95

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Gln Met Ser Leu Val Tyr Asp Pro Ala Ile Asn Glu Tyr Arg Asn Leu
      100              105              110

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Ala Gly Val Glu Thr Arg Val Pro Asn Phe Gly Ser Thr Arg Ala Phe
      115              120              125

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Ser His Lys Asn Pro Leu Lys Ser Asp Trp Cys Leu Gly Lys Leu Arg
      130              135              140

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Ala Ala Leu Glu Asp Met Gly Tyr Arg Asp Gly Asp Thr Met Phe Gly
      145              150              155              160

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```

Ala Pro Tyr Asp Phe Arg Tyr Ala Pro Pro Ser Pro Gly Gln Thr Ser
      165              170              175

```

```

Glu Val Tyr Ser Arg Tyr Phe Lys Glu Leu Met Glu Leu Val Glu Ala
      180              185              190

```

```

Ala Ser Glu Arg Thr Arg Lys Lys Ala Val Ile Leu Gly His Ser Phe
      195              200              205

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Gly Gly Met Val Ala Leu Glu Phe Val Arg Asn Thr Pro Pro Ala Trp
 210 215 220

Arg Arg Glu His Ile Glu Arg Leu Val
 225 230

<210> 5
 <211> 1217
 <212> DNA
 <213> Glycine max

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 ttcattacca ccaagaactc gatgattact tcaacactcc tgggggttgag acccggtgcc 180
 ctcaactttg ttccaccaac tctcttctct atctcaatcc tcgtctcaag catatcacccg 240
 gatacatggc acccctggta gattcattac aaaagcttgg ctacgctgat ggtgagactc 300
 tgtttggagc cccttatgac tttagatatg gtctagctgc tgaaggtoac ccttcacaag 360
 tgggttccaa gttcctcaaa gatctaaaga atttgataga agaagcaagc aattccaata 420
 atgggaagcc agtgatactt ctctcccaca gtttaggagg cctatttgtc ctacaactac 480
 taaatagaaa ccccccctct tggcgcaaaa aattcatcaa acacttcatt gctctttcag 540
 ctccatgggg tgggtgtata gacgaaatgt acacctttgc atctggcaac actttgggag 600
 tgcccctagt ggacccttta ttagtgaggg atgaacaaag aagctccgag agtaaccttt 660
 ggcttttgcc taacccaaaa atttttggtc ctcaaaaacc aatagtata actccaatta 720
 ggccttattc agctcatgac atggttgatt ttctaaaaga cattgggtttt cctgaagggg 780
 tttatcctta tgaaacacga attctaccct tgatagggaa cataaaagca ccacaagtgc 840
 ctataacttg tattatggga acgggagtgg gaaccttgga aacattggtt tatgggaaag 900
 gtgattttga tgaacggcca gaaatatcat atggggatgg tgatggaacg gtgaacttgg 960
 tgagcttggt ggcgcttcaa tcaactatga aagaggagaa aaatcaatac cttaaagtgg 1020
 ttaagataga tggggtgtct catacttcaa tacttaagga tgaagttgca ctaaatgaaa 1080
 tagtaggtga gattacttca attaatctc atgctgagct cggtttaagt aatttgtttt 1140
 cggggtaaat gatcagggtg tttgaacgac aattatagat tcgttgtctg caaattaaat 1200
 tttgtgtggg gaggttga 1217

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 <211> 381
 <212> PRT
 <213> Glycine max

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Phe Arg Leu Trp Phe Asp Ser Ser Val Ile Leu Ala Pro Phe Thr Gln
 20 25 30

Cys Phe Ala Glu Arg Met Thr Leu His Tyr His Gln Glu Leu Asp Asp
 35 40 45

Tyr Phe Asn Thr Pro Gly Val Glu Thr Arg Val Pro His Phe Gly Ser
 50 55 60

Thr Asn Ser Leu Leu Tyr Leu Asn Pro Arg Leu Lys His Ile Thr Gly
 65 70 75 80

Tyr Met Ala Pro Leu Val Asp Ser Leu Gln Lys Leu Gly Tyr Ala Asp
 85 90 95

Gly Glu Thr Leu Phe Gly Ala Pro Tyr Asp Phe Arg Tyr Gly Leu Ala

			100				105				110					
Ala	Glu	Gly	His	Pro	Ser	Gln	Val	Gly	Ser	Lys	Phe	Leu	Lys	Asp	Leu	
			115				120				125					
Lys	Asn	Leu	Ile	Glu	Glu	Ala	Ser	Asn	Ser	Asn	Asn	Gly	Lys	Pro	Val	
			130				135				140					
Ile	Leu	Leu	Ser	His	Ser	Leu	Gly	Gly	Leu	Phe	Val	Leu	Gln	Leu	Leu	
			145				150				155				160	
Asn	Arg	Asn	Pro	Pro	Ser	Trp	Arg	Lys	Lys	Phe	Ile	Lys	His	Phe	Ile	
			165				170				175					
Ala	Leu	Ser	Ala	Pro	Trp	Gly	Gly	Ala	Ile	Asp	Glu	Met	Tyr	Thr	Phe	
			180				185				190					
Ala	Ser	Gly	Asn	Thr	Leu	Gly	Val	Pro	Leu	Val	Asp	Pro	Leu	Leu	Val	
			195				200				205					
Arg	Asp	Glu	Gln	Arg	Ser	Ser	Glu	Ser	Asn	Leu	Trp	Leu	Leu	Pro	Asn	
			210				215				220					
Pro	Lys	Ile	Phe	Gly	Pro	Gln	Lys	Pro	Ile	Val	Ile	Thr	Pro	Ile	Arg	
			225				230				235				240	
Pro	Tyr	Ser	Ala	His	Asp	Met	Val	Asp	Phe	Leu	Lys	Asp	Ile	Gly	Phe	
			245				250				255					
Pro	Glu	Gly	Val	Tyr	Pro	Tyr	Glu	Thr	Arg	Ile	Leu	Pro	Leu	Ile	Gly	
			260				265				270					
Asn	Ile	Lys	Ala	Pro	Gln	Val	Pro	Ile	Thr	Cys	Ile	Met	Gly	Thr	Gly	
			275				280				285					
Val	Gly	Thr	Leu	Glu	Thr	Leu	Phe	Tyr	Gly	Lys	Gly	Asp	Phe	Asp	Glu	
			290				295				300					
Arg	Pro	Glu	Ile	Ser	Tyr	Gly	Asp	Gly	Asp	Gly	Thr	Val	Asn	Leu	Val	
			305				310				315				320	
Ser	Leu	Leu	Ala	Leu	Gln	Ser	Leu	Trp	Lys	Glu	Glu	Lys	Asn	Gln	Tyr	
			325				330				335					
Leu	Lys	Val	Val	Lys	Ile	Asp	Gly	Val	Ser	His	Thr	Ser	Ile	Leu	Lys	
			340				345				350					
Asp	Glu	Val	Ala	Leu	Asn	Glu	Ile	Val	Gly	Glu	Ile	Thr	Ser	Ile	Asn	
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<212>	DNA
<213>	Zea mays

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gtcgagacgc gcgtgcgcaa ttccggctcc tcccagggat tccagaagaa cccggagcac 420
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gacggcgaca cctgttccgg ggccccgtac gacctccgct acgccccgcc ggtgcccggc 540
cagccatcga ggtcttctcc ggctacttcc gtcggctggc cgagcctcgt cgaggacgcg 600
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gcgctggagt tcgtccggag cactcccatg gcgtggcgag acaggtacat caagcacctc 720
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gggtccaacc tgatgtacgt cccgacagtc agctcgctcg agcctgcctt taggcccgatg 840
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ccgctcgtgg tcaccgcgcg gaggaactac tccgcctacg acctggagga cctcctcgtc 960
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 <212> PRT
 <213> Zea mays

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 Arg Cys Arg Arg Glu Val Leu Leu His Pro Leu Val Leu Val Pro Gly
 35 40 45
 Leu Thr Cys Ser Glu Leu Asp Ala Arg Leu Thr Asp Ala Tyr Arg Pro
 50 55 60
 Phe Arg Ala Ala Cys Asp Glu Gly Glu Gly Leu Val Arg Leu Trp Thr
 65 70 75 80
 Asn Cys Ser Asp Leu Pro Ala His His Tyr Val Arg Cys Phe Met Glu
 85 90 95
 Gln Met Ala Leu Val Tyr Asp Pro Val Ala Asn Asp Tyr Arg Asn Leu
 100 105 110
 Pro Gly Val Glu Thr Arg Val Arg Asn Phe Gly Ser Ser Arg Gly Phe
 115 120 125
 Gln Lys Asn Pro Glu His Thr Thr Trp Ser Trp Cys Phe Glu Val Leu
 130 135 140
 Arg Asn Glu Leu Ala Arg Ala Gly Tyr Arg Asp Gly Asp Thr Leu Phe
 145 150 155 160

Gly Ala Pro Tyr Asp Leu Arg Tyr Ala Pro Pro Val Pro Gly Gln Pro
 165 170 175
 Ser Arg Ser Ser Pro Ala Thr Ser Val Gly Trp Pro Ser Leu Val Glu
 180 185 190
 Asp Ala Ser Arg Lys Asn Arg Gly Arg Lys Val Ile Leu Phe Gly His
 195 200 205
 Ser Phe Gly Gly Met Val Ala Leu Glu Phe Val Arg Ser Thr Pro Met
 210 215 220
 Ala Trp Arg Asp Arg Tyr Ile Lys His Leu Phe Leu Val Ala Pro Val
 225 230 235 240
 Pro Ala Glu Gly Phe Val Lys Pro Leu Gln Tyr Phe Val Ser Gly Ser
 245 250 255
 Asn Leu Met Tyr Val Pro Thr Val Ser Ser Leu Glu Pro Ala Phe Arg
 260 265 270
 Pro Met Trp Arg Thr Phe Glu Ser Ser Leu Val Asn Phe Pro Ser Pro
 275 280 285
 Ala Val Phe Gly Arg Arg Pro Leu Val Val Thr Ala Arg Arg Asn Tyr
 290 295 300
 Ser Ala Tyr Asp Leu Glu Asp Leu Leu Val Ala Val Gly Tyr Gly Ala
 305 310 315 320
 Gly Val Glu Pro Phe Arg Arg Arg Ala Val Pro Lys Met Ser Tyr Phe
 325 330 335
 Gln Ala Pro Met Val Pro Thr Thr Cys Met Asn Gly Val Gly Asn Asp
 340 345 350
 Thr Pro Glu Gln Leu Val Tyr Trp Asp Gly Asp Phe Asp Ala Thr Pro
 355 360 365
 Glu Ile Val Tyr Gly Asp Gly Asp Asn Ser Ile Asn Leu Val Ser Met
 370 375 380
 Leu Ala Phe Asp Glu Lys Met Arg Arg Gln Pro Glu Gln Asn Lys Val
 385 390 395 400
 Tyr Lys Ser Ile Lys Ile Arg Gly Ala Gln His Gly Thr Ile Val Thr
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 Asp Asp Thr Ala Leu Lys Arg Val Met His Glu Ile Leu Glu Ala Asn
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Arg Ser

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 <211> 1500
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 <213> Zea mays

<220>

<221> unsure
 <222> (536)
 <223> n=A, C, G, or T

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 acaagttcgt gtccgggctg gagcgggctcg cgtaccgcga cggcgagaac ctgttcggcg 480
 cgccctacga cttccggtac gccgtcgccc cgccaggcca cccgtcgagg gtccgngacg 540
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 tcacgaaaag ttgagataac aaattttcat cgtagcattg taaggaaata ggtggtaagc 1380
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 <212> PRT
 <213> Zea mays

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 20 25 30
 Val Val Val Val Pro Gly Tyr Ala Thr Asn Glu Leu Asp Ala Arg Leu
 35 40 45
 Thr Glu Leu Tyr His Pro Ser Ser Pro Arg Cys Ala His Lys Gly Lys
 50 55 60
 Gly Trp Phe Arg Leu Tyr Leu Asn Tyr Thr Ala Leu Glu Asp Ala Ala
 65 70 75 80
 Asp Val Arg Cys Phe Ala Glu Gln Met Ala Thr Ala Tyr Asp Ala Ala
 85 90 95
 Ser Asp Asp Tyr Arg Asn Ala Gln Gly Val Glu Thr Arg Val Pro Phe
 100 105 110
 Phe Gly Ser Thr Arg Ala Phe Arg Tyr Pro Asp Pro Asp Arg Arg Asn
 115 120 125

Phe 130	Ser	Tyr	Met	Asp	Lys	Phe 135	Val	Ser	Arg	Leu	Glu 140	Arg	Leu	Ala	Tyr
Arg 145	Asp	Gly	Glu	Asn	Leu 150	Phe	Gly	Ala	Pro	Tyr 155	Asp	Phe	Arg	Tyr	Ala 160
Val	Ala	Pro	Pro	Gly 165	His	Pro	Ser	Arg	Val 170	Ala	Asp	Ala	Phe	Phe 175	Gly
Arg	Leu	Arg	Arg 180	Leu	Val	Glu	Arg	Ala 185	Ser	Arg	Ala	Asn	Gly 190	Gly	Gly
Pro	Val	Thr 195	Ile	Val	Ala	His	Ser 200	Tyr	Gly	Gly	Thr 205	Val	Ala	His	Gln
Phe 210	Leu	Leu	Arg	Arg	Pro	Leu 215	Pro	Trp	Arg	Arg	Arg 220	Phe	Val	Arg	Arg
Phe 225	Val	Pro	Val	Ala	Ala 230	Pro	Trp	Gly	Gly	Val 235	Val	Leu	Gly	Met	Leu 240
Thr	Ile	Val	Ala	Gly 245	Asn	Asn	Leu	Gly	Leu 250	Pro	Phe	Val	Asp	Pro 255	Leu
Ala	Leu	Lys	Gly 260	Glu	Tyr	Arg	Ser	Leu 265	Gln	Ser	Ser	Leu	Trp 270	Pro	Leu
Pro	Asn 275	Pro	Asn	Ala	Phe	Arg	Ala 280	Gly	Gln	Pro	Leu 285	Val	Thr	Thr	Arg
Ser 290	Arg	Thr	Tyr	Thr	Ala	His 295	Asp	Met	Ala	Asp	Phe 300	Leu	Asp	Ala	Ile
Gly 305	Leu	Gly	Ala	Ala	Ile 310	Val	Pro	Tyr	Gln	Ser 315	Arg	Val	Leu	Pro	Leu 320
Phe	Arg	Glu	Leu	Pro 325	Ser	Pro	Arg	Val	Pro 330	Val	Ala	Cys	Val	Val 335	Gly
Val	Gly	Leu	Asp 340	Thr	Pro	Glu	Met	Leu 345	Ala	Tyr	Pro	Gly	Asp 350	Asp	Phe
Asp	Val	Thr 355	Pro	Met	Met	Val	Met 360	Gly	Asp	Gly	Asp 365	Gly	Leu	Val	Asn
Leu 370	Val	Ser	Leu	Leu	Ala	Val 375	Asp	Pro	Ala	Trp	Arg 380	Leu	Pro	Thr	Ala
Tyr 385	Phe	Arg	Met	Leu	Lys 390	Val	Arg	Asn	Val	Ser 395	His	Thr	Gly	Leu	Phe 400
Val	Asp	Asp	Ala	Ala 405	Leu	Ala	Val	Ile	Ile 410	Ser	Ala	Ile	Leu	Arg 415	Pro

Asn

$\langle 210 \rangle$	11
$\langle 211 \rangle$	1660

<212> DNA
<213> Zea mays

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<212> PRT
<213> Zea mays

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Val Ser Ala Asn Asn Phe His Pro Ile Phe Leu Val Ala Gly Val Ser
35 40 45
Cys Ser Asp Leu Glu Ala Arg Leu Thr Glu Glu Tyr Arg Pro Ser Val
50 55 60
Pro His Cys Gly Ala Met Lys Gly Lys Gly Trp Phe Gly Leu Trp Lys
65 70 75 80
Asn Ser Ser Glu Leu Leu Ser Arg Asp Tyr Val Gln Cys Phe Glu Glu
85 90 95
Gln Met Ser Leu Val Tyr Asp Pro Ala Ile Asn Glu Tyr Arg Asn Leu
100 105 110

Ala 115	Gly 120	Val 125	Glu 130	Thr 135	Arg 140	Val 145	Pro 150	Asn 155	Phe 160	Gly 165	Ser 170	Thr 175	Arg 180	Ala 185	Phe 190
Ser 130	His 135	Lys 140	Asn 145	Pro 150	Leu 155	Lys 160	Ser 165	Asp 170	Trp 175	Cys 180	Leu 185	Gly 190	Lys 195	Leu 200	Arg 205
Ala 145	Ala 150	Leu 155	Glu 160	Asp 165	Met 170	Gly 175	Tyr 180	Arg 185	Asp 190	Gly 195	Asp 200	Thr 205	Met 210	Phe 215	Gly 220
Ala 160	Pro 165	Tyr 170	Asp 175	Phe 180	Arg 185	Tyr 190	Ala 195	Pro 200	Pro 205	Ser 210	Pro 215	Gly 220	Gln 225	Thr 230	Ser 235
Glu 175	Val 180	Tyr 185	Ser 190	Arg 195	Tyr 200	Phe 205	Lys 210	Glu 215	Leu 220	Met 225	Glu 230	Leu 235	Val 240	Glu 245	Ala 250
Ala 185	Ser 190	Glu 195	Arg 200	Thr 205	Arg 210	Lys 215	Lys 220	Ala 225	Val 230	Ile 235	Leu 240	Gly 245	His 250	Ser 255	Phe 260
Gly 195	Gly 200	Met 205	Val 210	Ala 215	Leu 220	Glu 225	Phe 230	Val 235	Arg 240	Asn 245	Thr 250	Pro 255	Pro 260	Ala 265	Trp 270
Arg 205	Arg 210	Glu 215	His 220	Ile 225	Glu 230	Arg 235	Leu 240	Val 245	Leu 250	Val 255	Ala 260	Pro 265	Thr 270	Leu 275	Pro 280
Gly 215	Gly 220	Phe 225	Leu 230	Glu 235	Pro 240	Val 245	Arg 250	Asn 255	Phe 260	Ala 265	Ser 270	Gly 275	Thr 280	Asp 285	Ile 290
Leu 225	Tyr 230	Val 235	Pro 240	Ala 245	Thr 250	Thr 255	Pro 260	Leu 265	Ala 270	Thr 275	Arg 280	Ala 285	Met 290	Trp 295	Arg 300
Ser 235	Phe 240	Glu 245	Ser 250	Ala 255	Ile 260	Val 265	Asn 270	Phe 275	Pro 280	Ser 285	Pro 290	Ala 295	Val 300	Phe 305	Gly 310
Arg 245	Leu 250	Gln 255	Ala 260	Pro 265	Leu 270	Val 275	Val 280	Thr 285	Arg 290	Glu 295	Arg 300	Asn 305	Tyr 310	Ser 315	Ala 320
Ser 255	Ala 260	His 265	Asp 270	Met 275	Glu 280	Arg 285	Phe 290	Leu 295	Ala 300	Ala 305	Val 310	Gly 315	Ser 320	Gly 325	Glu 330
Ala 265	Ala 270	Glu 275	Pro 280	Phe 285	Arg 290	Arg 295	Arg 300	Ala 305	Val 310	Pro 315	Lys 320	Met 325	Gly 330	Ser 335	Phe 340
Ala 275	Ala 280	Pro 285	Met 290	Val 295	Pro 300	Met 305	Thr 310	Tyr 315	Ile 320	Ser 325	Gly 330	Val 335	Gly 340	Asn 345	Arg 350
Thr 285	Pro 290	Leu 295	Arg 300	Leu 305	Val 310	Phe 315	Trp 320	Gly 325	Glu 330	Asp 335	Phe 340	Asp 345	Ala 350	Ala 355	Pro 360
Glu 295	Val 300	Ala 305	Ala 310	Tyr 315	Gly 320	Asp 325	Arg 330	Asp 335	Gly 340	Lys 345	Ile 350	Asn 355	Leu 360	Ile 365	Ser 370
Val 305	Leu 310	Ala 315	Phe 320	Glu 325	Lys 330	Glu 335	Met 340	Arg 345	Arg 350	Gln 355	Pro 360	Glu 365	Gln 370	Lys 375	Lys 380
Gln 315	Phe 320	Lys 325	Ser 330	Ile 335	Lys 340	Ile 345	Asn 350	Lys 355	Ala 360	Gln 365	His 370	Ser 375	Thr 380	Ile 385	Val 390
Thr 325	Asp 330	Asp 335	Phe 340	Ala 345	Leu 350	His 355	Arg 360	Val 365	Ile 370	Gln 375	Glu 380	Ile 385	Val 390	Glu 395	Ala 400

Asn Asn Gln Lys Ile Pro Ser
435

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<211> 1332
<212> DNA
<213> Glycine max

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ctaataccag gtaacggagg gaaccaacta gaagcaagg tgaaccaatca gtacaagccc 180
tctactttca tctgccaatc atggtaccct ctcatcaaga aaaagaatgg atgggtcaga 240
ctttggtttg attccagtggt catacttgct cctttcactc aatgctttgc cgaacgcattg 300
acccttcatt accaccaaga actcgatgat tacttcaaca ctccctggggg tgagaccgg 360
gtccctcact ttggttccac caactctctt ctctatctca atcctcgtct caagcatatc 420
accggataca tggcaccctt ggtagattca ttacaaaagc ttggctacgc tgatggtgag 480
actctgtttg gagcccttta tgactttaga tatggtctag ctgctgaagg tcacccttca 540
caagtggggt ccaagttcct caaagatcta aagaatttga tagaagaagc aagcaattcc 600
aataatggga agccagtgat acttctctcc cacagtttag gaggcctatt tgtcctacaa 660
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tcagctccat ggggtggtgc tatagacgaa atgtcacct ttgcatctgg caacctttg 780
ggagtgcctc tagtggaacc tttattagtg agggatgaac aaagaagctc cgagagtaac 840
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gtggttaaga tagatggggt gtctcacta tcaatactta aggatgaagt tgcactaaat 1260
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ttttcggggg aa 1332

<210> 14
<211> 443
<212> PRT
<213> Glycine max

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Thr Val Thr Val Val Val Val Met Leu Ser Leu Leu Cys Thr Cys Gly
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Ala Ser Asn Leu Asp Pro Leu Ile Leu Ile Pro Gly Asn Gly Gly Asn
35 40 45
Gln Leu Glu Ala Arg Leu Thr Asn Gln Tyr Lys Pro Ser Thr Phe Ile
50 55 60
Cys Glu Ser Trp Tyr Pro Leu Ile Lys Lys Lys Asn Gly Trp Phe Arg
65 70 75 80
Leu Trp Phe Asp Ser Ser Val Ile Leu Ala Pro Phe Thr Gln Cys Phe
85 90 95
Ala Glu Arg Met Thr Leu His Tyr His Gln Glu Leu Asp Asp Tyr Phe
100 105 110

Ala Glu Leu Gly Leu Ser Asn Leu Phe Ser Gly
435 440

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<210> 15
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<400> 15

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Val Val Thr Met Thr Ser Met Cys Gln Ala Val Gly Ser Asn Val Tyr
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Pro Leu Ile Leu Val Pro Gly Asn Gly Gly Asn Gln Leu Glu Val Arg
35 40 45

Leu Asp Arg Glu Tyr Lys Pro Ser Ser Val Trp Cys Ser Ser Trp Leu
50 55 60

Tyr Pro Ile His Lys Lys Ser Gly Gly Trp Phe Arg Leu Trp Phe Asp
65 70 75 80

Ala Ala Val Leu Leu Ser Pro Phe Thr Arg Cys Phe Ser Asp Arg Met
85 90 95

Met Leu Tyr Tyr Asp Pro Asp Leu Asp Asp Tyr Gln Asn Ala Pro Gly
100 105 110

Val Gln Thr Arg Val Pro His Phe Gly Ser Thr Lys Ser Leu Leu Tyr
115 120 125

Leu Asp Pro Arg Leu Arg Asp Ala Thr Ser Tyr Met Glu His Leu Val
130 135 140

Lys Ala Leu Glu Lys Lys Cys Gly Tyr Val Asn Asp Gln Thr Ile Leu
145 150 155 160

Gly Ala Pro Tyr Asp Phe Arg Tyr Gly Leu Ala Ala Ser Gly His Pro
165 170 175

Ser Arg Val Ala Ser Gln Phe Leu Gln Asp Leu Lys Gln Leu Val Glu
180 185 190

Lys Thr Ser Ser Glu Asn Glu Gly Lys Pro Val Ile Leu Leu Ser His
195 200 205

Ser Leu Gly Gly Leu Phe Val Leu His Phe Leu Asn Arg Thr Thr Pro
210 215 220

Ser Trp Arg Arg Lys Tyr Ile Lys His Phe Val Ala Leu Ala Ala Pro
225 230 235 240

Trp Gly Gly Thr Ile Ser Gln Met Lys Thr Phe Ala Ser Gly Asn Thr
245 250 255

Leu Gly Val Pro Leu Val Asn Pro Leu Leu Val Arg Arg His Gln Arg
260 265 270

[illegible]

Thr	Ser	Glu 275	Ser	Asn	Gln	Trp	Leu 280	Leu	Pro	Ser	Thr	Lys 285	Val	Phe	His
Asp	Arg 290	Thr	Lys	Pro	Leu	Val 295	Val	Thr	Pro	Gln	Val 300	Asn	Tyr	Thr	Ala
Tyr 305	Glu	Met	Asp	Arg	Phe 310	Phe	Ala	Asp	Ile	Gly 315	Phe	Ser	Gln	Gly	Val 320
Val	Pro	Tyr	Lys	Thr 325	Arg	Val	Leu	Pro	Leu 330	Thr	Glu	Glu	Leu	Met 335	Thr
Pro	Gly	Val	Pro 340	Val	Thr	Cys	Ile	Tyr 345	Gly	Arg	Gly	Val	Asp 350	Thr	Pro
Glu	Val	Leu 355	Met	Tyr	Gly	Lys	Gly 360	Gly	Phe	Asp	Lys	Gln 365	Pro	Glu	Ile
Lys	Tyr 370	Gly	Asp	Gly	Asp	Gly 375	Thr	Val	Asn	Leu	Ala 380	Ser	Leu	Ala	Ala
Leu 385	Lys	Val	Asp	Ser	Leu 390	Asn	Thr	Val	Glu	Ile 395	Asp	Gly	Val	Ser	His 400
Thr	Ser	Ile	Leu	Lys 405	Asp	Glu	Ile	Ala	Leu 410	Lys	Glu	Ile	Met	Lys 415	Gln
Ile	Ser	Ile	Ile 420	Asn	Tyr	Glu	Leu	Ala 425	Asn	Val	Asn	Ala	Val 430	Asn	Glu